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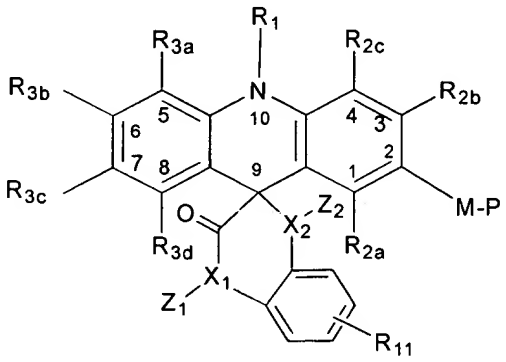
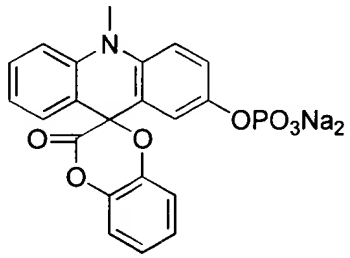
PATENT & TRADEMARK OFFICE

21. The chemiluminescent substrate of claim 20 having the following structure:



wherein R11 is a single or multiple substitution, each substituent of which is selected from the group consisting of hydrogen, -R, substituted or unsubstituted aryl (ArR or Ar), halides, nitro, sulfonate, sulfate, phosphonate, $-CO_2H$, -C(O)OR, cyano (-CN), -SCN, -OR, -SR, -SSR, -C(O)R, -C(O)NHR, ethylene glycol, or polyethyelene glycol.

22. The chemiluminescent substrate of claim 21 having the following structure:



23. The chemiluminescent substrate of claim 2 wherein said chemiluminescent moiety Lumi is an acridinium compound having the following structure:

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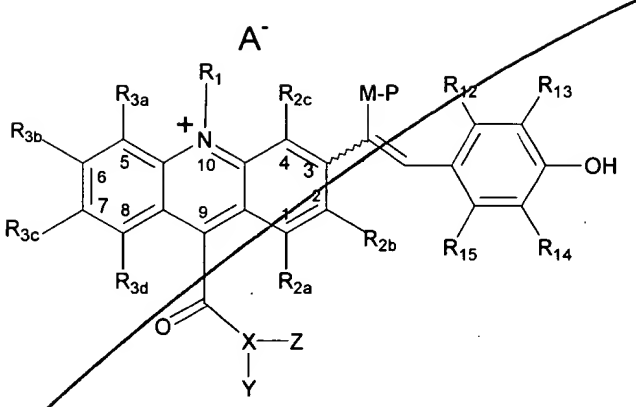

5 wherein, R_1, R_{2a-c}, R_{3a-d}, A^-, M, P, X, Y, and Z are as defined
6 in claim 4; R12, R13, R14 and R15 are the same or different and
7 are selected from the group consisting of hydrogen, -R,
8 hydroxyl, amino, halides, nitro, nitroso, sulfonate, sulfate,
9 phosphonate, $-CO_2H$, cyano (-CN), -SCN, -OR, -SR, -SSR, -C(O)R,
10 and -C(O)NHR.

1 24. The chemiluminescent substrate of claim 23 wherein any
2 adjacent two groups of R12 to R15 can form one or more
3 additional fused hydrocarbon aromatic rings or heteroaromatic
4 rings with or without substitutions, said rings selected from
5 the group consisting of benzene, naphthlene, pyridine,
6 thiophene, furan, and pyrrole.

1 25. The chemiluminescent substrate of claim 23 having the
2 following structure:

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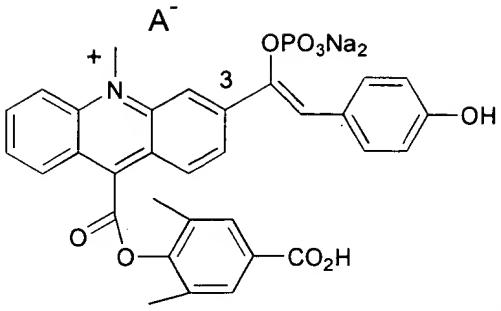
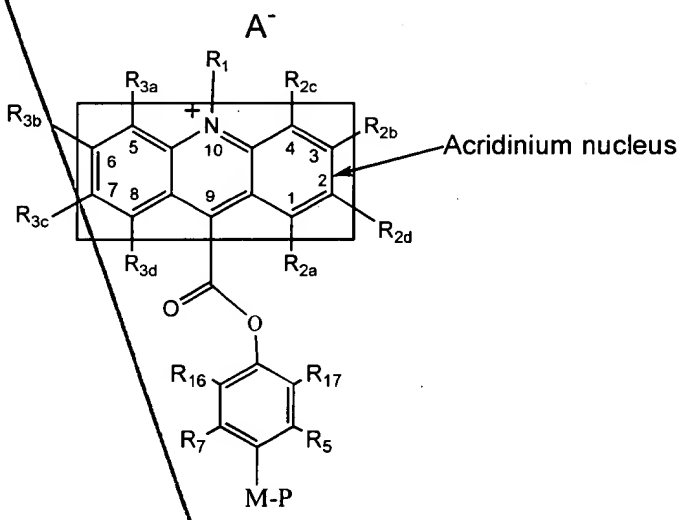


26. The chemiluminescent substrate of claim 2 wherein said chemiluminescent moiety Lumi is an acridinium compound having the following structure:



wherein, R_1, R_{2a-c}, R_{3a-d}, R_5, R_7, A^-, M, and P are as defined in claim 4;

R_{2d} is as defined for R_{2a-c} and R_{3a-d};

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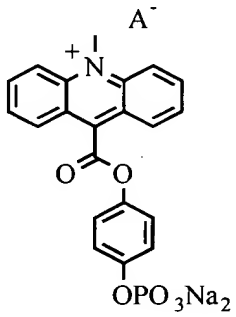
R_{16} and R_{17} are the same or different, and are selected from the group consisting of hydrogen, methyl, alkyl with low molecular weight, and halides.

27. The chemiluminescent substrate of claim 26 wherein R_{16} and R_{17} are different and one of them is hydrogen.

28. The chemiluminescent substrate of claim 26 wherein both R_{16} and R_{17} are hydrogen.

29. The chemiluminescent substrate of claim 26 having the following structure:



30. An enzymatic reaction

$$\text{Lumi-M-P} \xrightarrow{\text{HE}} \text{Lumi-M} + \text{P}$$

wherein:

a. Lumi-M-P is a chemiluminescent substrate of a hydrolytic enzyme

b. HE is a hydrolytic enzyme

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c. Lumi-M is a chemiluminescent enzymatic product having properties different from Lumi-M-P

31. The enzymatic reaction of claim 30, wherein said properties are selected from the group consisting of emission wavelength, quantum yield, light emission kinetics, net charge distribution, dipole moment, π-bond orders, free energy, hydrophobicity/ hydrophilicity, solubility, and affinity.

32. The enzymatic reaction of claim 30, wherein HE is selected from the group consisting of phosphatases, glycosidases, peptidases, proteases, esterases, sulfatase and guanidinobenzoatase.

33. An apparatus for light detection which is capable of maximizing the distinction between Lumi-M from Lumi-M-P in the reaction of claim 30.

34. The apparatus of claim 33, selected from the group consisting of a luminometer, charge-coupled device camera, X-ray film, and high speed photographic film.

35. The apparatus of claim 33, wherein the maximization of the distinction can be achieved by employing optical filters.

36. The apparatus of claim 33, wherein the maximization of the distinction can be achieved by employing a red sensitive photo multiplier tube in a luminometer or back-thinned cooled charge

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coupled device for detecting longer wavelength emitting Lumi-M or Lumi-M-P.

37. The apparatus of claim 33, wherein the maximization of the distinction can be achieved by employing a blue sensitive photo multiplier tube in a luminometer for detecting the shorter wavelength emitting Lumi-M or Lumi-M-P.

Cont a1

38. A method of enhancing the distinction between Lumi-M from Lumi-M-P in the reaction of claim 30 by treating the post enzymatic reaction mixture with alkali followed by hydrogen peroxide.

39. A method for the detection and/or quantitation of a hydrolytic enzyme in a sample comprising the steps of:

a. providing an enzymatically hydrolyzable chemi-luminescent Lumi-M-P selected from claims 1-25 capable of emitting light at a first wavelength maximum when chemically treated;

b. contacting said Lumi-M-P compound with said sample containing said enzyme to allow the enzymatic reaction of claim 30 to occur for the generation of said Lumi-M capable of emitting light at a second wavelength maximum when chemically treated;

c. detecting said emitted lights selectively or individually as an indication of the presence and/or the amount of said enzyme.

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40. An assay method for the detection and/or quantitation of an analyte in a sample comprising the steps of:

a. combining said sample with at least a member of binding pair labeled with a hydrolytic enzyme to form a binding complex;

b. providing an enzymatically hydrolyzable chemi-luminescent Lumi-M-P selected from claims 1- 25 capable of emitting light at a first wavelength maximum when chemically treated;

c. contacting said Lumi-M-P compound with said binding complex to allow the enzymatic reaction of claim 30 to occur for the generation of said Lumi-M capable of emitting light at a second wavelength maximum when chemically treated;

d. detecting said emitted lights selectively or individually as an indication of the presence and/or amount of said analyte.

41. A method for the detection and/or quantitation of a hydrolytic enzyme in a sample comprising the steps of :

a. providing an enzymatically hydrolyzable chemi-luminescent Lumi-M-P selected from claims 26-29 capable of emitting light within a first time interval when chemically treated;

b. contacting said Lumi-M-P compound with said sample containing said enzyme to allow the enzymatic reaction of claim 30 to occur for the generation of said Lumi-M

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capable of emitting light within a second time interval when chemically treated; and

c. detecting said emitted lights within said first time interval or within said second time interval to discern the change in the light intensity as an indication of the presence and/or the amount of said enzyme.

Cont a'

42. An assay method for the detection and/or quantitation of an analyte in a sample comprising the steps of:

a. combining said sample with at least a member of binding pair labeled with a hydrolytic enzyme to form a binding complex;

b. providing an enzymatically hydrolyzable chemiluminescent Lumi-M-P selected from claims 26-29 capable of emitting light within a first time interval when chemically treated;

c. contacting said Lumi-M-P compound with said binding complex to allow the enzymatic reaction of claim 30 to occur for the generation of said Lumi-M capable of emitting light within a second time interval when chemically treated; and

d. detecting said emitted lights within said first time interval or within said second time interval to discern the change in the light intensity as an indication of the presence and/or amount of said analyte.

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